**HIGHLIGHTED STUDENT RESEARCH**

# Environment, phylogeny, and photosynthetic pathway as determinants of leaf traits in savanna and forest graminoid species in central Brazil

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## Abstract

Leaf traits are closely linked to plant responses to the environment and can provide important information on adaptation and evolution. These traits may also result from common ancestry, so phylogenetic relationships also play an important role in adaptive evolution. We evaluated the effects of the closed forest environment (gallery forest) and the open savanna environment (cerrado) on the selection of leaf traits of graminoid species. The two plant communities differ in light, nutrients, and water availability, which are important drivers in the selection and differentiation of these traits. We also investigated the functional structure and the role of phylogeny in the functional organization of species, considering leaf traits. Patterns of leaf trait variation differed between forest and savanna species suggesting habitat specialization. Wider and longer leaves, with higher values of specific leaf area, chlorophyll, and nitrogen, seem to be an advantage for graminoid species growing in forest environments, while thicker leaves, with higher values of leaf dry-matter content and carbon, benefit species growing in savanna environments. We found few phylogenetic signals related to leaf traits in each environment. Therefore, the functional similarity that the gallery forest and cerrado graminoid species share within their group is independent of their phylogenetic proximity. Environmental filters affect the functional structure of communities differently, generating communities with trait values that are more distant than expected by chance in cerrado (functional dispersion), and closer than expected by chance in the gallery forest (functional convergence).

**Keywords** Functional diversity · Grasses · Open habitats · Shaded habitats · Cerrado

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## Introduction

Studies of the diversity patterns, distribution, and functioning of plant communities should take into account species functional traits, particularly those relevant to the specific process being studied (Tilman et al. 1997; Díaz et al. 2016). Functional traits are biochemical, morphological, physiological, phenological, or developmental characteristics of species (Violle et al. 2007) that significantly influence their fitness (Lavorel and Garnier 2002; Reich et al. 2003) as well as processes within the community (Fukami et al. 2005). One of the main objectives of comparative plant ecology is to understand how functional traits differ among species and to what extent these differences have adaptive value (Reich et al. 2003). Therefore, trait differentiation among phylogenetically closely related species occupying contrasting habitats strongly suggests adaptive divergence (Ackerly et al. 2000; Hoffmann and Franco 2003; Baughman et al. 2019).

It is well established that functional traits can provide a more ecologically meaningful assessment of a plant community assembly. Because of their ability to functionally respond to the biotic and abiotic environment, leaf traits have been extensively used in ecological studies seeking to understand ecosystem processes (e.g.,Hulshof and Swenson 2010; Lavorel et al. 2011; Pringle et al. 2011; Coble et al. 2017). Leaf traits are closely linked to the main responses and effects of plants on the environment, serving as indicators of ecosystem functioning (Craine et al. 2001). Leaf traits also strongly influence plant competitive ability (Kunstler et al. 2016) and growth (Niklas et al. 2007), since leaves capture and assimilate light energy and $CO_2$ in photosynthesis, and generate essential organic compounds for plant metabolism and development.

Abiotic filters, such as light, water, and nutrient availability, can give rise to convergence of functional traits, causing species clustering across multiple niche dimensions and affecting community structuring and phylogenetic composition (Fukami et al. 2005). Thus, it is not surprising that plants thriving in humid shady environments, such as in the forest understory, differ in a suite of traits related to leaf structure, gas exchange and nutrient status in comparison with plants that succeed in open sunny habitats (Cayssials and Rodríguez 2013; Petter et al. 2016).

By integrating the species evolutionary history, phylogenetic information can provide useful insights in the interpretation of ecological processes related to niche selection and of the adaptive and functional significance of traits underlying plant community structure and composition (Cavender-Bares et al. 2009; Solofondranohatra et al. 2018). Functional trait syndromes in grasses are determined by phylogenetic relationships, environmental variation, and the photosynthetic pathway (Edwards et al. 2010). For instance, the expansion of grasses and other graminoid species into savanna environments is associated with the pre-existence of the $C_4$ photosynthetic pathway in these clades. This pathway involves major changes in leaf anatomy that are fine-tuned to adjustments in leaf metabolic pathways, which ultimately result in a higher $CO_2$ gain per unit of transpired water in areas exposed to high irradiances, high temperatures, and drought conditions (Jacobs et al. 1999; Sage et al. 2012).

The Cerrado biome in central Brazil is dominated by savanna-like vegetation on well-drained, deep dystrophic soils (Ribeiro and Walter 2008). The typical savanna physiognomy is locally known as cerrado sensu stricto (hereafter referred as cerrado). The upper canopy of cerrado (10–60% tree cover) consists of 3–8-m tall deciduous, semi-deciduous and evergreen trees. Most deciduous species remain leafless for a short period of time, around 1 month at the end of the dry season (Lenza and Klink 2006). Linear strips of gallery forest (with 50–90% tree cover, 20–30-m tall) occur along the numerous small rivers and streams flowing through the region (Ribeiro and Walter 2008). The gallery forest trees are essentially evergreen, and the large majority of species retain their leaves year-round (Funch et al. 2002). Peak leaf production of cerrado tree species occurs in the late dry season, while for gallery forest species, leaf flushing happens earlier, and the annual radial growth rate is about twofold higher in gallery forest than in cerrado (Rossatto et al. 2009). Soils of cerrado are nutrient-poor and the upper soil layers dry out during the dry season (Haridasan 2008; Hoffmann et al. 2009; Geiger et al. 2011), whereas gallery forests thrive in soils with more nutrients and water available (Correia et al. 2001; Bueno et al. 2018). Wind speed and air temperature are higher, and relative humidity is lower in the cerrado than in the gallery forests (Hoffmann et al. 2012).

In the Cerrado, grasses (Poaceae) and sedges (Cyperaceae) are the most abundant plants in the ground layer, and these families are especially species-rich (Flora do Brasil 2020; Jardine et al. 2020). Graminoid species are also commonly found in the gallery forest understory (Chaves and Soares-Silva 2012) and are one of the main components of savanna formations (Veldman et al. 2015; Archibald et al. 2019), where this group is usually more diverse (Amaral et al. 2017). The graminoid floras of gallery forests and cerrado differ in species composition (Flora do Brasil 2020). Cerrado holds a continuous species-rich graminoid stratum, whereas in the gallery forest grasses and sedges are not as species-rich and have a scattered distribution (Chaves and Soares-Silva 2012; Souza et al. 2020). Cerrado and gallery forest trees are well-characterized functionally (for instance,Hoffmann and Franco 2003; Hoffmann et al 2009; Rossatto et al. 2009, 2013). However, comparative studies of functional diversity, trait relationships, and photosynthetic pathway of the graminoid cerrado and gallery forests communities are lacking.

In this study, we evaluated the leaf trait variation and functional structure of native graminoid species (Poaceae and Cyperaceae) that are typical of savanna (cerrado) and gallery forest habitats. We expected that much less light would be available in the gallery forest understory than in the cerrado (Hoffmann and Franco 2003), since the gallery forest has a greater tree cover (Ribeiro and Walter 2008). In addition, differences in soil nutrients and water availability between the savanna and forest would affect the selection and differentiation of species adapted to each of these environments (Cayssials and Rodríguez 2013; Rossatto et al. 2013; Gallaher et al. 2019). We selected leaf traits known to respond to these environmental conditions and hypothesized that forest and savanna graminoid species would be associated with particular leaf trait combinations related to habitat specialization. For instance, trait combinations related to drought resistance or water use efficiency (e.g., smaller, and thicker leaves) in cerrado species and to light use efficiency (e.g., larger leaves with higher chlorophyll concentration)

in gallery forest species. We expected differences in photosynthetic pathway between the two habitats, with $C_3$ species dominating in the gallery forest and $C_4$ species in the cerrado, as found studies elsewhere that compared savanna and forest environments (Cayssials and Rodríguez 2013; Solofondranohatra et al. 2018). We also investigated the role of phylogeny in structuring the functional composition of the two graminoid communities and used functional diversity indexes to assess community structure.

## Materials and methods

### Study area and species selection

The study was carried out in a gallery forest and a cerrado (savanna formation) in the National Park of Brasília in the northwest portion of the Federal District, Brazil (15° 35′–15° 45′ S, 47° 55′–48° 55′ W), 900 to 1000 m a.s.l. According to Köppen's classification, the climate of the study site is Aw, with two well-defined seasons: a rainy summer (October–April) and a dry winter (May–September), with an annual mean temperature of 21 °C and an annual mean rainfall of 1500 mm. The edaphic characteristics of gallery forest are distinct from the cerrado. In general, gallery forest soils have greater availability of nutrients and water (Correia et al. 2001), while cerrado soils are poorer (Haridasan 2008; Hoffmann et al. 2009; Geiger et al. 2011).To confirm our hypothesis about the difference in light availability, we evaluated the degree of canopy openness by hemispherical photography at 10 different points in the study areas. Photographs were taken using a digital camera (Nikon D80) with a fish-eye lens (Nikon FC-E9) horizontally levelled, aimed at the zenith and north oriented, at 1.0 m from the ground. Photographs were taken under overcast sky conditions during the end of the rainy season. The photographs were analyzed using the software Gap Light Analyzer, Version 2.0 (Frazer et al. 1999). The canopy openness averaged 67.5% ($n = 10$, SD = 26.2%) in the cerrado and 16.4% ($n = 10$, SD = 1.9%) in the gallery forest.

We selected 10 abundant graminoid species in gallery forest and 14 in cerrado (Table 1). The selected species are widely distributed in the Cerrado biome and each species is found in only one of the two vegetation types sampled here (Silva and Nogueira 1999; Chaves and Soares-Silva 2012; Flora do Brasil 2020).

### Measurement of leaf traits

Ten replicates of each leaf trait (one leaf per plant/individual) were obtained from individuals at least 5 m apart (Pérez-Harguindeguy et al. 2013). We collected mature leaves, fully expanded and free of signs of herbivory or

**Table 1** Graminoid species from gallery forest and cerrado used in this study and their habitat

| Species | Habitat |
| --- | --- |
| Poaceae | |
| *Aristida riparia* Trin | Cerrado |
| *Aristida torta* (Nees) Kunth | Cerrado |
| *Axonopus siccus* (Nees) Kuhlm | Cerrado |
| *Echinolaena inflexa* (Poir.) Chase | Cerrado |
| *Eragrostis polytricha* Nees | Cerrado |
| *Paspalum foliiforme* S. Denham | Cerrado |
| *Paspalum gardnerianum* Nees | Cerrado |
| *Paspalum plicatulum* Michx | Cerrado |
| *Paspalum polyphyllum* Nees | Cerrado |
| *Paspalum reduncum* Nees ex Steud | Cerrado |
| *Paspalum stellatum* Humb. & Bonpl. ex Flüggé | Cerrado |
| *Setaria parviflora* (Poir.) Kerguélen | Cerrado |
| *Trachypogon spicatus* (L.f.) Kuntze | Cerrado |
| *Tristachya leiostachya* Nees | Cerrado |
| *Hildaea pallens* (Sw.) C. Silva & R. P. Oliveira | Gallery forest |
| *Homolepis glutinosa* (Sw.) Zuloaga & Soderstr | Gallery forest |
| *Lasiacis ligulata* Hitchc. & Chase | Gallery forest |
| *Olyra taquara* Swallen | Gallery forest |
| *Oplismenus hirtellus* (L.) P. Beauv | Gallery forest |
| *Otachyrium seminudum* Hack. ex Send. & Soderstr | Gallery forest |
| Cyperaceae | |
| *Calyptrocarya irwiniana* T. Koyama | Gallery forest |
| *Rhynchospora exaltata* Kunth | Gallery forest |
| *Scleria latifolia* Sw | Gallery forest |
| *Scleria microcarpa* Nees ex Kunth | Gallery forest |

pathogens during the peak of the rainy season (February), in the morning when temperatures were cooler, to minimize water loss and interference in attribute values. Leaves were packed in sealed, moist plastic bags with damp paper that were transported to the laboratory in a cooler with ice, to preserve the water saturation of the leaves until the time of the measurements. We measured leaf width (cm), leaf length (cm), leaf thickness (mm), specific leaf area (SLA, $cm^2\ g^{-1}$), leaf dry-matter content (LDMC, g), total chlorophyll levels (ChlAB, $\mu g\ cm^{-2}$), carotenoid levels (Car, $\mu g\ cm^{-2}$), and leaf percentages of carbon (%C) and nitrogen (%N) (Appendix A: Table A1).

Leaf width was measured at the median portion of the leaf, and length was measured from the base of the leaf blade to the apex. Leaf thickness was measured with the aid of a digital caliper, avoiding the central rib to reduce sampling variation. SLA is the one-sided area of a fresh leaf, divided by its oven-dry mass. The fresh leaf area ($cm^2$) was measured with a Laser Leaf Area Meter (CI-202). Therefater, the samples were oven-dried at 70 °C for 72 h and weighed in a Semi-Analytical Balance (AD200, Marte, Bel 0.001–210 g)

to obtain leaf dry mass (Pérez-Harguindeguy et al. 2013). LDMC is the oven-dry mass of a leaf, divided by its water-saturated fresh mass (Pérez-Harguindeguy et al. 2013). All measurements were performed in the same leaf for each replicate.

For chlorophyll measurements, 2 ml of *N,N*-dimethylfor-mamide (DMF) was added to each amber-colored Eppendorf tube and the tube was stored in a refrigerator at 4 °C 1 day before the sample collection. On the collection day, the Eppendorf tubes were placed in a cooler containing ice and taken to the field. In the field, a 3.9 mm-diameter leaf disc was taken from each replica with the aid of a cork borer, immediately placed in an Eppendorf tube, and returned to the cooler. The samples were transported to the laboratory and stored in a refrigerator at 4 °C for 48 h (Moran and Porath 1980). After this time, the absorbances of the samples were read at the wavelengths of 663.8 nm, 646.8 nm and 480 nm, using a spectrophotometer (Thermo Spectronic—Genesys 2 PC) to determine the concentrations of chlorophyll A, B and carotenoids. The wavelengths and equations for calculating chlorophyll A, B and carotenoid concentrations were based on Wellburn's (1994) study. For the chlorophyll measurements, we used other leaves (one per plant) from the same individuals sampled for the first measurements.

To measure leaf %C and %N on a leaf-mass basis, a mixed sample was collected from 10 leaves (one per plant) from the same individuals sampled for the previous measurements, and from this single mixed sample, two samples were taken for each species. Leaf carbon and nitrogen were determined by the ultra-combustion method at 950 °C in a TruSpec CHN628 elemental analyzer (Leco Corp., St. Joseph, Michigan, USA). Prior to analysis, the leaf samples were oven-dried at 60 °C for 72 h, ground to powder, and then returned to a container with silica gel for 12 h to remove any residual moisture. About 0.1 g of ground material from each sample was used.

Each species was classified as $C_3$ or $C_4$ based on the available literature (Bruhl and Wilson 2007; Osborne et al. 2014).

## Statistical analyses

The traits of species from gallery forest and cerrado were compared by ANOVA test followed by Holm correction to account for multiple model comparisons ($P < 0.05$). Data normality and homogeneity of variances was assessed using the Shapiro–Wilk test. To satisfy normality assumptions, we used $\log_{10}$ transformations. A cluster analysis was performed to evaluate the functional proximity between all species. To construct the dendrogram we used the "dist.ktab" function in the "ade4" package (Dray and Dufour 2007). This function, which is a mixed-variables coefficient of distance, generates the distance matrix from a mixed matrix, as in our case,

where we consider the leaf traits (continuous variables), and also the photosynthetic pathway, which is a binary variable. To generate grouping of species by functional proximity from the distance matrix, we used the "hclust" function in the "stats" package (R Development Core Team 2013), using the "average" method, which generates a UPGMA grouping. We transformed the dendrogram into an object of phylo class using the "as.phylo" function in the "picante" package (Kembel et al. 2010). To plot the trait values in the functional tree, we used the "phylo4d" function in the "phylobase" package (Hackathon et al. 2011), to generate an object of the phylo4d class from the previously generated functional dendrogram, and the functional-traits matrix of the species. Then we used the function "table.phylo4d" in the "adephylo" package (Jombart and Dray 2010), enabling the "scale" argument, which standardizes the variables to plot the functional dendrogram along with the values of each measured trait. The functional dendrogram was built from a matrix with the means of the trait values of all species. Species groupings were visually identified in the dendrogram.

To understand how the gallery forest and cerrado environments affect the functional structure of graminoid species, we calculated three continuous measures of functional diversity: Functional diversity index (FD; Petchey and Gaston 2002, 2006), mean pairwise functional distance (MFD), and mean nearest functional distance (MNFD), which are measurements based on metrics used in phylogenetic diversity, including the phylogenetic diversity index (PD; Faith 1992), mean pairwise phylogenetic distance (MPD), and mean nearest taxon distance (MNTD; Webb 2000). Here, we replaced the phylogenetic tree with the functional tree for analysis, since both can be represented by a similar data structure, any index applied to a phylogenetic tree can be applied to a functional dendrogram (Pavoine and Bonsall 2011). For this analysis we used the same functional dendrogram created by the UPGMA cluster from the matrix of functional attributes and a matrix of presence and absence of species in the communities. We used null models to determine if the coexisting species similarity was due to chance. For all metrics we calculated the standardized effect sizes against null communities (SES). The observed functional proximity was compared to the expected random pattern using the ses.pd, ses.mpd and ses.mntd functions in the "picante" package (Kembel et al. 2010), using 999 randomizations. Standardized effects values greater than zero indicate functional clustering and values less than zero indicate functional overdispersion (Webb et al. 2002; Vamosi et al. 2009).

A phylogenetic independence analysis was also performed (Phylogenetic independent contrasts, PICs), to detect whether there was a phylogenetic signal in leaf traits and whether phylogenetically closer species shared similar trait values, as closely related species are more likely to be ecologically similar, due to phylogenetic conservation of niche

(Losos 2008). We used Blomberg's *K* test, which compares the variance of PICs with what would be expected in a Brownian motion (random) model (Blomberg et al. 2003). We used the "phylosignal" function in the "picante" package (Kembel et al. 2010), using the matrix with the $log_{10}$ transformed trait values, and the phylogenetic tree of the species, with 999 randomizations. This analysis was performed separately for the gallery forest and the cerrado, to analyze how the phylogenetic proximity of the species of each environment influences the functional similarity of each group, since the similarity of attributes between species may be related to their phylogenetic proximity (Losos 2008). A significant *P* value ($< 0.05$) in this analysis means that a phylogenetic signal exists, and that phylogenetically close species are more similar than random pairs of species. All analyses were performed using R version 3.6.0 (R Development Core Team 2013).

To generate the phylogenetic tree used in the PIC analysis, we used sequence data obtained from the GenBank database (Clark et al. 2016). Species that did not have sequences in GenBank were sequenced. We used the trnL–trnF intergenic spacer region, as it was the most frequently sequenced marker deposited in GenBank. Total DNA was extracted from fresh leaves, using a rapid small-scale DNA extraction protocol (mini-CTAB) (Doyle and Doyle 1987) with modifications according to Borsch et al. (2003). Samples were amplified by Polymerase Chain Reaction—PCR (Mullis and Faloona 1987). Extraction and PCRs were conducted at the Plant Molecular Biology Laboratory at the University

of Brasília, and PCR products were purified and sequenced by Macrogen (Seoul, Korea). The sequences generated were deposited in GenBank.

The sequences were automatically aligned using MUSCLE software, and then manually in PhyDE software (v. 0.9971) (Müller et al. 2006). jModelTest 2.1.7 software (Guindon and Gascuel 2003; Darriba et al. 2012) was used to statistically select the best evolution model of aligned nucleotides. Phylogenetic inferences were made using Bayesian analysis in MrBayes v. 3.2.6 software (Ronquist et al. 2012) using the phylogenetic research platform CIPRES (Miller et al. 2010). We used Tracer software v. 1.5 (Rambaut and Drummond 2011) to analyze the reliability of the Bayesian analyses, and Figtree v.1.3.1 software (Rambaut 2009) to build the phylogenetic tree.

## Results

Cerrado and gallery forest graminoid species differed significantly for most leaf traits. Leaves of gallery forest species were wider, longer and thinner, with higher values of SLA, chlorophyll and %N. In contrast cerrado species had thicker leaves, with higher values of LDMC and carbon (Fig. 1). Cerrado and forest graminoid species did not differ in total carotenoid levels ($F_{1,23} = 0.57$, $P = 0.45$).

The functional dendrogram (Fig. 2) showed two species clusters. Open-habitat (cerrado) species clustered in one group, while the closed-habitat (gallery forest) species
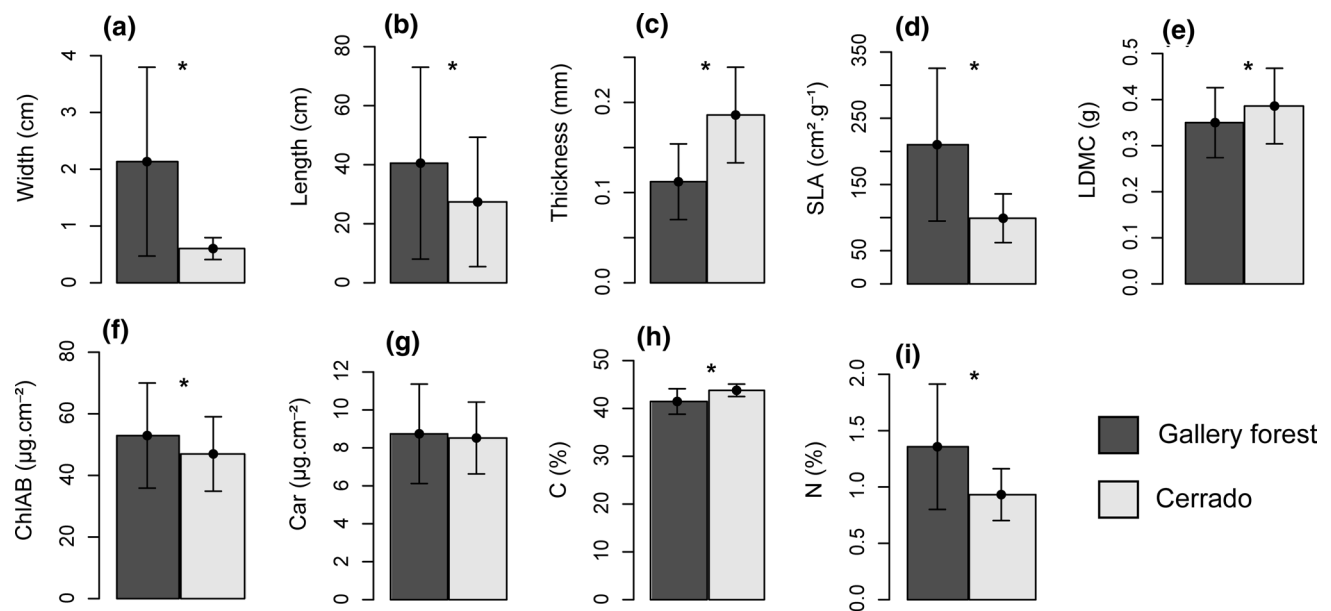


**Fig. 1** Means and standard deviation of leaf traits measured in graminoid species from cerrado ($n = 14$, 10 replicates) and gallery forest ($n = 10$, 10 replicates). **a** Width—Leaf width, **b** length—leaf length, **c** thickness—leaf thickness, **d** SLA—specific leaf area, **e** LDMC— leaf dry-matter content, **f** ChlAB—total chlorophyll, **g** car—carotenoids, **h** C(%)—leaf carbon percentage, **i** N(%)—leaf nitrogen percentage. Asterisks show significant differences after Holm correction ($P < 0.05$)
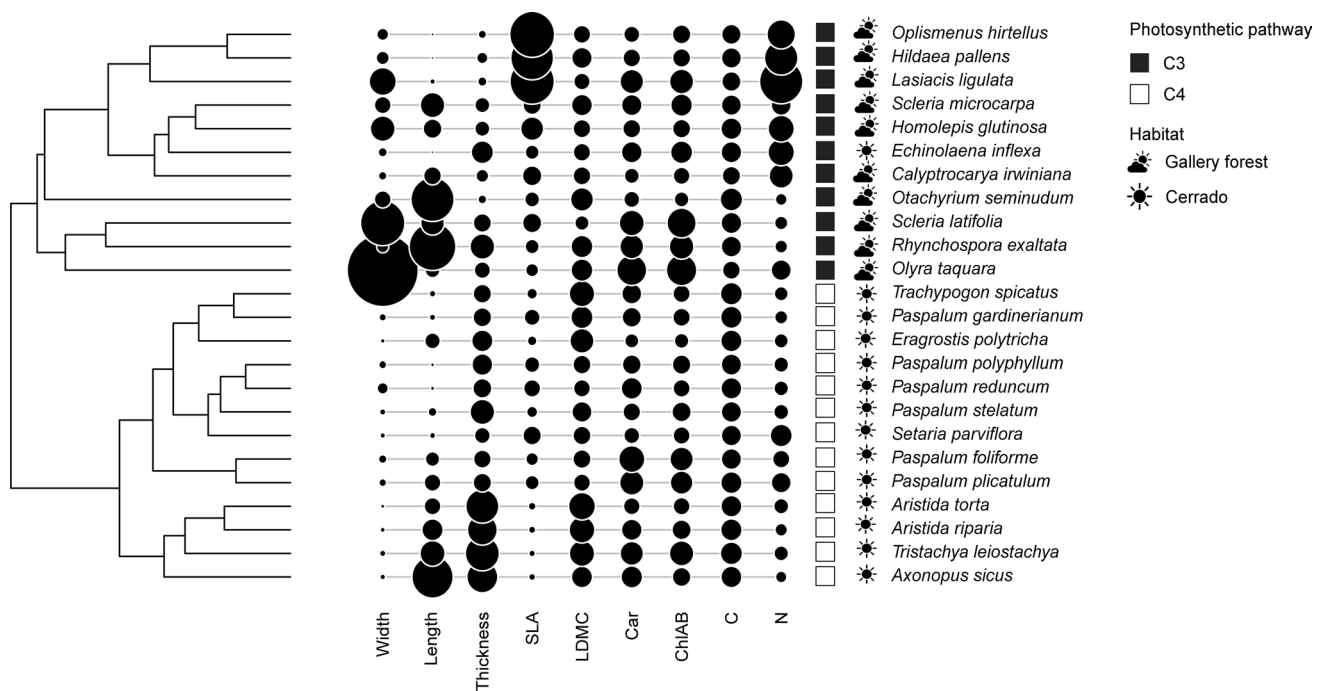
**Fig. 2** Functional dendrogram of the graminoid species from gallery forest and cerrado. The traits are represented by the mean values for each species. Sizes of circles represent the proportional trait values for each species: the larger the circle, the higher the trait value in relation to the other species. The dendrogram shows two clusters of species with similar leaf-trait values. One cluster contains the graminoid species from open, sunny habitats (cerrado), and the other cluster contains the species that thrive in closed, shaded habitats (gallery forest). The only exception was the cerrado grass *Echinolaena inflexa*, which clustered with the forest-species group

clustered in another group. The only exception to this pattern of segregation was the open-habitat grass *Echinolaena inflexa*, which grouped with gallery-forest species. *Echinolaena inflexa* was also the only species that did not share the same photosynthetic pathway present in the other open-habitat graminoid species, being a $C_3$ species, while all other cerrado species had the $C_4$ pathway (Fig. 2).

Significant phylogenetic signals were found only for four leaf traits and only among cerrado species. SLA and LDMC showed a strong phylogenetic signal, while width and thickness showed a moderate phylogenetic signal (K-statistic values closer to 1 suggest a strong phylogenetic signal) (Table 2). Thus, phylogenetically closer graminoid species in the cerrado tended to have similar values for these four traits. We did not detect a significant phylogenetic signal for any of the leaf traits for the gallery-forest species (Table 2). The traits with non-significant *P* values are, therefore, randomly distributed in the phylogeny.

When compared with the null model, the species of cerrado showed a functional divergence pattern for all metrics (SES values less than 0, and $P < 0.05$), that is, they were functionally more distant than expected by chance (Table 3). For the gallery-forest species, we did not observe any significant functional structure, meaning that these traits are randomly assembled in this community (Table 3).

## Discussion

We found a clear distinction in leaf-trait values and photosynthetic pathway between the graminoid species of gallery forest and cerrado. In the forest environment, shading can limit photosynthesis, generating a selection pressure for leaf attributes that increase light absorption by leaves and favor grasses with $C_3$ photosynthetic pathway (Valladares et al. 2016). Thus, graminoid species from the gallery forest understory have longer and wider but thinner leaves. The higher SLA values of gallery-forest graminoid species may be associated with greater availability of resources, such as water and nutrients, in this environment than in the cerrado (Hoffmann et al. 2009; Geiger et al. 2011; Parron et al. 2011). In contrast, in the open environment of cerrado, one of the main limiting factors is water (Sfair et al. 2016) rather than light availability. Relative to the forest environment, warmer, drier, and windier conditions prevail in the cerrado (Hoffmann et al. 2012). Here, traits that increase water-use efficiency (i.e., carbon gain per unit water loss) under high irradiances, such as the $C_4$ photosynthetic pathway, and changes in leaf morphology that can facilitate heat exchange between leaves and the surrounding air, increase mechanical strength and reduce

**Table 2** Phylogenetic signal (PIC) test on leaf traits of graminoid species from cerrado and gallery forest

| Functional trait | $K$ | Observed variance of PICs | Mean of random variances of PICs | $P$ |
|---|---|---|---|---|
| *Cerrado* | | | | |
| Width | 0.54 | 1.979 | 3.831 | **0.037** |
| Length | 0.36 | 2.298 | 3.103 | 0.238 |
| Thickness | 0.56 | 1.626 | 3.347 | **0.043** |
| SLA | 0.82 | 2.193 | 6.394 | **0.005** |
| LDMC | 0.98 | 0.572 | 1.589 | **0.004** |
| Car | 0.21 | 1.869 | 1.477 | 0.777 |
| ChlAB | 0.39 | 0.898 | 1.08 | 0.339 |
| C | 0.63 | 0.032 | 0.048 | 0.165 |
| N | 0.34 | 2.168 | 2.717 | 0.379 |
| *Gallery forest* | | | | |
| Width | 0.25 | 8.573 | 1.047 | 0.466 |
| Length | 0.25 | 3.013 | 2.919 | 0.567 |
| Thickness | 0.36 | 1.928 | 3.356 | 0.132 |
| SLA | 0.34 | 5.653 | 7.066 | 0.332 |
| LDMC | 0.26 | 0.35 | 0.407 | 0.381 |
| Car | 0.34 | 1.027 | 1.47 | 0.271 |
| ChlAB | 0.45 | 0.778 | 1.383 | 0.117 |
| C | 0.38 | 0.085 | 0.133 | 0.211 |
| N | 0.59 | 2.171 | 4.867 | 0.06 |

Significance values at $P < 0.05$ are shown in bold

**Table 3** Species-based standardized effect sizes (SES) of functional diversity (FD), mean functional distance (MFD) and mean nearest functional distance (MNFD), based on leaf traits in graminoid species present in gallery forest and cerrado communities

| Community | SES.FD | SES.MFD | SES.MNFD |
|---|---|---|---|
| Gallery forest | 1.617 (0.955) | 0.137 (0.474) | 1.900 (0.977) |
| Cerrado | − 3.619 (**0.001**) | − 6.600 (**0.001**) | − 2.345 (**0.021**) |

Significance values are in parentheses. Values at $P < 0.05$ are shown in bold

water loss, such as small, narrow and sclerophyllous leaves (lower SLA and higher thickness), are important.

The biochemical $CO_2$-concentrating mechanism of $C_4$ plants, generating internally a high $CO_2$ environment, results in higher rates of $CO_2$ fixation per unit water lost, and lower allocation of nitrogen to Rubisco. Therefore, $C_4$ cerrado graminoid species would be expected to have lower leaf %N but higher nitrogen and water—use efficiency compared to $C_3$ gallery-forest species that thrive in soils with higher nitrogen levels (Hoffmann et al. 2009). By supporting high photosynthetic rates and nitrogen use efficiencies in high-irradiance *N*-limited environments, the $C_4$ pathway would also facilitate fast biomass accumulation after fire events that are common in the cerrado (Edwards et al 2010).

In addition, in the cerrado, where nutrients and water are less available (Haridasan 2008; Sfair et al. 2016), species would be under strong selection to allocate more resources to structural defenses to protect leaf tissues from herbivory and pathogens (Coley et al. 1985), thus favoring graminoid species that have leaves with lower SLA and higher levels of LDMC and %C. LDMC is also negativelly correlated with leaf turgor loss point (Liu and Osborne 2015), suggesting that cerrado grasses are more drought tolerant than forest species. Thus, the divergence in several leaf functional traits between cerrado and forest graminoid species is an adaptive response to the different selective pressures affecting these two contrasting vegetation types. Functional distinctions were closely associated to photosynthetic routes, with the environment exerting ecological selection pressures for the $C_3$ or $C_4$ photosynthetic pathway.

Photosynthetic pathways are related to traits such as leaf length and width, and plant height (Oyarzabal et al. 2008; Gallaher et al. 2019), further promoting the strong convergence in leaf traits observed here. Furthermore, the evolution of Kranz anatomy, as a means to support $C_4$ photosynthesis, has the potential to drive leaf anatomical changes, including those associated with interveinal distances and leaf thickness (Ehleringer and Monson 1993). Evidence of the potential direct influence of photosynthetic pathway on the evolution of leaf traits is seen in the $C_3$ species, *Echinolaena inflexa*. Despite being typical of the cerrado, it lacks the $C_4$ pathway and its associated anatomical traits. Overall, its leaf traits clustered with the $C_3$ forest species in the functional dendrogram (Fig. 2). Additional work is needed to discern patterns of selection in shaping the interplay among photosynthetic pathway, leaf allometry and environment. Of particular importance is the need to assess the potential for the environment to act directly as a selective agent shaping leaf trait spectra, or indirectly by favoring certain traits, such as photosynthetic type, which then influences leaf traits through anatomical constraint.

The relative wide variation in leaf width and length observed in gallery forest is due the presence of species from different subfamilies in Poaceae. For example, the more basal Bambusoideae (e.g., *Olyra taquara*), is known to have wider leaves, while species belonging to the subfamily Panicoideae are more recent in origin and are characterized by leaves of intermediate sizes (Gallaher et al. 2019). Cyperaceae species (*Rhynchospora exaltata*, *Scleria latifolia* and *Scleria macrocarpa*) are also characterized by having leaves that are wider in shape than grass leaves.

The higher chlorophyll levels in gallery-forest graminoid species may result from the tendency of the leaf chlorophyll content to increase as light availability decreases (Hallik et al. 2009), probably to maximize absorption of the available light and to reach light compensation at lower irradiances (Rossatto et al. 2018). In environments with

high irradiance levels, the rate of chlorophyll decomposition is also higher (Burkholder 1936; Kowalewska and Szymczak 2001), which would explain the lower levels of leaf chlorophyll in species of cerrado. Carotenoid levels did not differ significantly between the species of gallery forest and cerrado. The lack of differentiation in this trait between the two environments may result from the dual role of carotenoid pigments. By expanding the wavelength range of light that is able to drive photosynthesis they can serve to enhance the overall efficiency of photosynthetic light reactions in shaded forest environments. However, they can also provide photoprotection by dissipating excessive light energy in high-irradiance environments (Demmig-Adams and Adams 1996), as in the cerrado (Franco et al. 2007).

Species with similar functional traits are often phylogenetically close (Losos 2008). When a strong phylogenetic signal is detected in functional traits, environmental filters are probably selecting for phylogenetically close species, causing phylogenetic clustering. Here, the graminoid species that separated into different functional groups with respect to leaf traits were not phylogenetically close within their group, except for the three species of Cyperaceae, which separated from Poaceae, probably because they belong to a different family (Appendix A: Fig. A1). The analyses of phylogenetic independence (PICs) detected a phylogenetic signal in only four traits and only for the open-environment species. This result came from the relationship of species that are mostly from the same subfamily (Panicoideae) in cerrado, and because all species in cerrado were $C_4$ (except *Echinolaena inflexa*), being more related phylogenetically. Therefore, for graminoid species of both gallery forest and cerrado, most leaf traits are independent of phylogenetic proximity, showing that the environment is one of the main factors shaping these attributes.

Gallery forest species are more similar to each other, and the cerrado species are more similar to each other. However, this does not mean that the same mechanisms were shaping leaf trait distribution within each environment. In the leaf traits studied here, the graminoid species of cerrado are functionally more distant than expected by chance. This suggests that species coexistence has been strongly influenced by competitive interactions, generating functional divergence and niche segregation, selecting for more-distant trait values during community assembly. Environmental heterogeneity may also play a role, given the patchiness of resource distribution of savanna landscapes in central Brazil (Franco et al. 2014). The gallery-forest environment seems to have a random effect in selecting leaf traits in graminoid species, since the values for these traits did not show deviations from randomness, suggesting, therefore, no effect of the gallery forest environment. Studies are needed to address more completely the interplay between environmental heterogeneity and competition in driving the functional assembly of the graminoid communities of gallery forest.

Our results emphasize that the assemblages of graminoid plants of gallery forest and cerrado differ in species composition and leaf functional traits that are associated to photosynthetic pathway. The trait differences result from evolutionary divergence and deterministic processes selecting similar traits even in phylogenetically distant species. These attributes allowed the two groups of graminoid species to adapt to environments with large differences in light, water availability and nutrients.

**Author contribution statement** EJA and CBRM conceived and designed the experiments. EJA conducted fieldwork, collected the data, and performed the experiments. EJA and VR generated sequencing data and molecular analyses, EJA, CBRM and ACF analyzed the data. EJA, CBRM and ACF wrote the manuscript. All authors contributed critically to data interpretation and manuscript reviewing and gave final approval for publication.

## Declarations

**Conflict of interest** The authors declare that they have no conflict of interest.

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